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Microplastic ingestion by small coastal fish in the northern Baltic Sea, Finland

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A B S T R A C T

Microplastic (MP) ingestion by four species of small coastal fish from the northern Baltic Sea was investigated. The digestive tract contents of 424 specimens, caught across eight sampling sites along the Finnish coastline were analysed for the occurrence of MP ingestion. MP were found in 38 fish individuals (9% of sampled fish). Specimens from the urban area of Helsinki displayed the highest prevalence of ingested plastics (27.5%). No relationship was found between the size or species of the fish and the presence of ingested MP particles nor the amount of MP in seawater. The comparison to a previous study conducted using the same research methods indicates that the ingestion of MP is more common in coastal fish than in offshore fish in the northern Baltic Sea.

1. Introduction

Marine litter is a vast global concern as litter is commonly found in the seas worldwide (Barnes et al., 2009; Bergmann and Klages, 2012; Galgani et al., 2000; Galgani et al., 1996; Ramirez-Llodra et al., 2011). The bulk of litter in marine environments is clearly dominated by plastics (Derraik, 2002; Moore, 2008), and most plastic particles are defined as microplastics (MP), i.e. particles smaller than 5 mm in size (Arthur et al., 2009). More restrictive definitions have recently been proposed for MP, that separate debris <5 mm into additional size categories: MP (1–5 mm), ultrafine (1 μm – 1 mm) and nanoplastics (1 nm – 1 μm), to account for differences in the accuracy and scope of detection methods (Provencher et al., 2017).

Marine animals, such as mammals, seabirds, turtles and fish may ingest both larger plastic debris and MP (e.g. Caron et al., 2018; Cartraud et al., 2019; Nelms et al., 2019; Rummel et al., 2016). Also, marine invertebrates are at a high risk of plastic contamination (Walkinshaw et al., 2020). Fish may ingest MP either intentionally if plastic particles resemble prey or other food (Lusher et al., 2013), or accidentally during their normal feeding activity (Rummel et al., 2016). Also, MP may accumulate in fish via the ingestion of MP contaminated prey (Batel et al., 2016; Nelms et al., 2018). Ingestion of MP can lead to adverse health effects on fish (Barboza et al., 2018; Batel et al., 2016) as MP may contain harmful chemicals, such as phthalates, bisphenol A (BPA), polybrominated diphenyl ethers (PBDE) and tetrabromobisphenol A (TBBPA) (Talsness et al., 2009). MP can also absorb harmful substances, such as persistent organic pollutants (POPs), from sea water (Mato et al., 2001). These chemicals may be transferred from MP to fish (Browne et al., 2007; Gassel et al., 2013) and potentially disturb their growth, survival and reproduction (Hutchinson et al., 1994; McKinley et al., 2011; Muirhead et al., 2006). It has also been suggested that MP may cause a false feeling of satiation and intestinal blockage in early life stages of fish (Foekema et al., 2013).

Coastal areas are important nursery habitats for several fish species and are most vulnerable due to high anthropogenic pressure (Sundblad et al., 2013); however, there is very little data yet from MP ingestion by coastal fish. In the present study, MP ingestion by bleak, (*Alburnus alburnus*), three-spined stickleback (*Gasterosteus aculeatus*), perch (*Perca fluviatilis*) and roach (*Rutilus rutilus*) was studied on the coast of Finland. Of the species included in this study, perch and roach also have value for recreational fishing. The research focused on the active uptake of particles >100 μm which has previously been studied in the offshore areas of the northern Baltic Sea (Budimir et al., 2018). The aim of the present study was to apply the same research methods to investigate MP ingestion by the coastal fish in sites under different human pressures. The hypothesis was that coastal fish are more exposed to MP than offshore fish (e.g. Browne et al., 2011; Harris, 2020); therefore, coastal fish may exhibit a higher prevalence of ingested MP.

Previous studies on MP ingestion by the species included in this study are scarce, and most of them have focused on freshwater ecosystems (Faure et al., 2015, Horton et al., 2018, Kusmierek and Popiolek, 2020). Exceptions are a study from the offshore areas of the Baltic Sea where

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the gastrointestinal tracts (GITs) of 355 sticklebacks were analysed (Budimir et al., 2018), and a study from the coastal waters of China where the GITs and gills of six perch were analysed (Zhu et al., 2019). Both Budimir et al. (2018) and Zhu et al. (2019) found only fibres, no plastic particles in stickleback or perch.

2. Materials and methods

2.1. Fish sampling

Fish samples were collected with a beach seine at eight sampling sites along the Finnish coastline in May–June 2017 (Fig. 1). The sampling sites were chosen based on their vicinity of different potential MP sources, such as highways, harbors and industry. Tvärminne, located in a nature conservation area, was chosen as a reference site.

The fish were terminated immediately after being caught by cutting the spinal cord with scissors, and were then stored in resealable zip-top bags at −20 °C for later analysis. A total of 424 fish of roughly similar size were chosen for the analyses. The selected species displayed common species in the northern Baltic coastal areas: bleak, three-spined stickleback, perch and roach (Table 1, Fig. 2). The average length of all studied fish was 7.8 ± 3.1 cm and the total wet weight 4.2 ± 4.4 g (mean ± SD). The four selected species display different feeding behaviors. Bleak feeds mostly on the water surface, three-spined stickleback and perch are both benthic and pelagic feeders, and roach feeds mostly on the benthos (Yrjölä et al., 2015; Table 1).

2.2. Water sampling

Water samples for MP analyses were taken at the same time and at the same locations as the fish samples, except for Tvärminne, where no water sample was taken. A pump device developed by Talvitie et al. (2015) was used for sampling. At each sampling location, water was pumped from the sea and filtered through two filters (100 and 300 μm plankton net, diameter approx. 70 mm), as described by Talvitie et al. (2015). The filtered sample volume varied between 60 and 515 L for 100–300 μm and 1000 L (or 2000 L on one occasion) for >300 μm depending on the amount of organic matter at the sampling location. After filtering, each filter was placed in a clean petri dish with tweezers, and the samples were stored at −20 °C.

2.3. Sample processing and analyses

To prevent sample contamination, all laboratory equipment was carefully rinsed with Milli-Q water, and all chemical solutions were filtered through a 0.7 μm glass microfiber filter (Whatman GF/F) before use. Sample processing was conducted in a fume hood. To monitor the amount of contamination in the fish samples, blank samples were prepared. Blank samples were empty centrifuge tubes (50 mL, polypropylene (PP), VWR Finland) that went through the same digestion

Fig. 1. Sampling sites along the Finnish coastline, northern Baltic Sea. Two of the sampling sites were situated in Helsinki.

Table 1
Studied fish species; number of specimens, length, weight, estimated age and feeding type.

Species name	Number of specimens	Length (Average ± SD, min, max)	Weight (Average ± SD, min, max)	Average age based on average length	Type of feeding based on average age
Bleak	127	9.0 ± 1.6, min 5.5, max 12.2	4.7 ± 2.5, min 0.8, max 12.9	~2–3 years	Pelagic; zooplankton and insects
Three-spined stickleback	120	6.1 ± 0.5; min 4,9, max 7,0	2.2 ± 0.6; min 0,9, max 3.6	~3 years	Benthic and pelagic; invertebrates, fish eggs, small fish
Perch	94	7.3 ± 1.1; min 5,2, max 11,2	3.6 ± 2.2; min 1,4, max 13,9	~1 year	Benthic and pelagic; macroinvertebrates, small fish
Roach	83	9.0 ± 1.8; min 5.0, max 12.8	6.9 ± 4.3; min 1,0, max 20.1	~3–4 years	Benthic; invertebrates

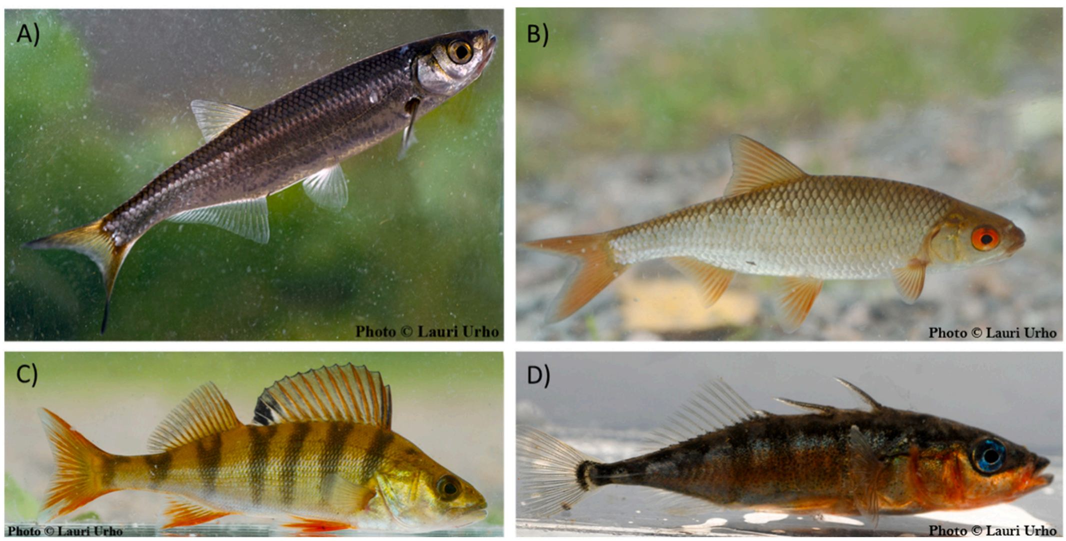

Fig. 2. Photos of the studied species: A) bleak (*Alburnus alburnus*), B) roach (*Rutilus rutilus*), C) perch (*Perca fluviatilis*) and D) three-spined stickleback (*Gasterosteus aculeatus*).

process as the fish samples.

The frozen fish were thawed at room temperature. The total length and weight of each fish were recorded before dissecting. Dissecting scissors were used to carefully remove the GITs, which were placed in 50 mL centrifuge tubes (50 mL, PP, VWR Finland), one GIT per tube. The GITs were digested using the method developed by Budimir et al. (2018). Briefly, 10 mL of 1 M NaOH and 5 mL of SDS (5 g/L) were added to the centrifuge tubes per approximately 1 *g* of fish tissue. The samples were incubated at 50 °C for 48 h. After the incubation, each sample was filtered through a 100 μm mesh size filter (plankton net, diameter approx. 47 mm). The walls of the centrifuge tube were carefully rinsed with 96% ethanol and Milli-Q water, and the rinsing liquids were filtered. Then, the funnel and the filter with the residue were thoroughly rinsed with 96% ethanol and Milli-Q water with the vacuum suction on. Lastly, the filter was placed in a clean petri dish and studied under a stereomicroscope (Leica Wild M8). Particles were identified as plastics by melting them with a hot needle. The ingested MP were categorized by size and color (Provencher et al., 2017).

Out of the water samples collected, the data from 300 μm and the 100 μm mesh-sized filters were pooled for the analyses, as the lowest size fraction of MP in the fish samples was >100 μm. The filters were thawed at room temperature and particles were identified as plastics under a stereomicroscope as described above.

2.4. Data analysis

Statistical analyses were performed with IBM SPSS Statistics. The frequency of MP occurrence was calculated as the percentage of individual fish within a species and within a location. The Kruskal-Wallis test was used to compare the frequency of occurrence of MP between fish species and between sampling locations. The Bonferroni test was used for multiple comparisons of different sampling locations. Logistic regression was used to test whether the length or the weight of the fish had an effect on the occurrence of MP. Spearman's correlation was used to test whether there was a relationship between the MP in fish and in the water samples of the same sampling locations.

A total of 5 clear plastic particles were found in the procedural blanks ($n = 95$) indicating contamination. Therefore, the mean value (0.05) for the MP found in the blank samples was subtracted from each clear plastic particle ($n = 20$) that was found in the fish to exclude the effect of contamination in further analyses.

3. Results

3.1. Microplastics in fish samples

Out of the 424 fish examined, 51 MP were found in the GITs of 38 fish, representing 9% of all studied individuals. In fish that had ingested MP, the number of items varied between 1 and 5 particles per fish (1.34 ± 0.71). After correcting the data based on the observed contamination found in the procedural blanks as described above, the total amount of clear MP in fish was 19, and the total amount of all plastic particles was 50. The ingested MP represented a variety of colors, with off/white-clear being the most common (78%). Other colors observed were red-pink, yellow and black. The size of plastics (largest diameter) ranged from 184 to 2592 μm (Table 2).

As the contamination of the airborne fibres in the samples was high, (98 fibres/54 blank samples), fibres were not included in the further

Table 2
Number of ingested MP categorized by size and color.

	1–5 mm	1 μm-1 mm	Total
Off/white-clear	5.7	33.2	39
Black	0	1	1
Red-pink	1	4	5
Yellow	0	5	5
Total	6.7	43.2	50

analyses.

There was a significant difference in the frequency of fish with MP between the sampling locations (Kruskal-Wallis test, F = 4.055, df = 8, $p < 0.001$). MP were found in 27.50% of the fish from the sampling site Kivinokka, situated in the urban metropolitan area of the city of Helsinki. The second highest frequency of ingestion (16.7%) was found in fish from the sampling site Porvoo. In other sampling sites, the MP prevalence was under 10%. The lowest frequency of ingestion was found in Pori (4.3%) (Fig. 3). The frequency of fish with MP was significantly higher in Kivinokka, Helsinki, than in other locations (Bonferroni test, $p = 0.001$–0.014) except for Porvoo ($p > 0.05$). Porvoo did not differ from any of the other sampling locations. There was no statistically significant difference between the other locations of Munkkiniemi, Kotka, Vaasa, Pori, Parainen and Tvärminne ($p > 0.05$).

Three-spined stickleback displayed the highest frequency of MP ingestion across all species (Table 3). The frequency of ingestion for bleak and perch was slightly lower, and for roach the lowest. No statistically significant difference was found between the fish species (Kruskal-Wallis test, $p > 0.05$). Further, there was no relationship between the size of the fish and the presence of ingested MP (logistic regression for length and weight, $p > 0.05$).

3.2. Microplastics in water samples

MP concentration in water samples was 16.2 ± 11.2 MP m^{-3} (mean ± SD) (Fig. 3). A total of 33 MP was found ranging in size between 140 and 2370 μm. Most of the MP were off/white-clear (81.8%). Other colors observed were blue–purple, red–pink and yellow. No statistically significant relationship was found between the amount of MP in fish and in water samples in the same sampling locations (Spearman's correlation, $p > 0.05$).

4. Discussion

The prevalence of coastal fish with ingested MP varied between 4.3

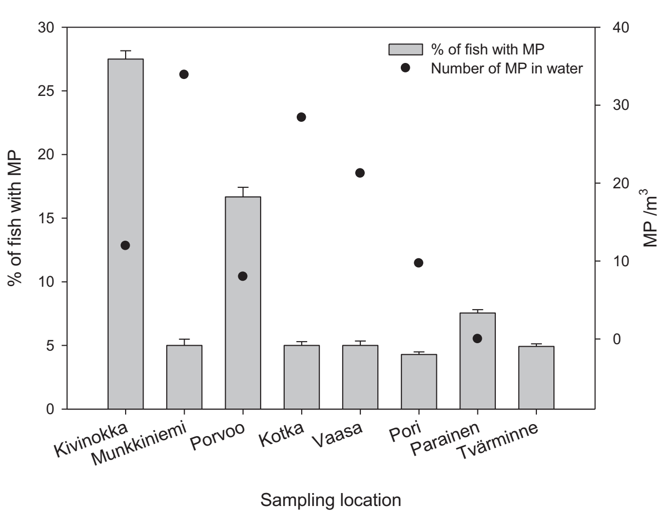

Fig. 3. Frequency of fish with ingested MP (grey columns, average + SD) and number of MP in water m^{-3} (black circles) across all sampling locations.

Table 3
Frequency of occurrence of ingested MP by the studied fish species.

Fish	Frequency of occurrence (%)	Number of plastic items	
		Mean (n; ±sd)	Range
Three-spined stickleback	12.5	0.2 ± 0.6	0–5
Bleak	10.2	0.2 ± 0.5	0–3
Perch	8.5	0.08 ± 0.3	0–1
Roach	2.4	0.03 ± 0.3	0–2

and 27.5% of the fish, depending on the sampling location. The share of fish with ingested MP was found to be higher than what was found in a previous study from the offshore areas of the northern Baltic Sea where the prevalence was only 1.8% for herring ($n = 164$), 0.9% for sprat ($n = 154$) and 0% for three-spined stickleback ($n = 355$) (Budimir et al., 2018). In contrast, the present study found 12.5% of the coastal three-spined sticklebacks ($n = 120$) to have MP in their GITs. The methods applied for extracting and identifying MP in these two studies were the same, thus allowing comparisons between results. Based on the results of this study, the ingestion of microplastics appears to be more common in coastal fish than in offshore fish. Coastal fish are likely more exposed to MP than fish occupying offshore areas, which is also consistent with previous studies (Barnes et al., 2009; Browne et al., 2011; Desforges et al., 2014) as most of the plastic litter in marine environments originates from land-based sources (Gewert et al., 2017; Jambeck et al., 2015).

In other studies from the Baltic Sea, the prevalence of MP in fish has varied between 4.9 and 22.3% (Beer et al., 2018; Lenz et al., 2016; Ogonowski et al., 2019; Rummel et al., 2016). While the results of the present study are comparable with the results of Budimir et al. (2018), other studies from the Baltic Sea have been conducted using different methods and thus cannot be directly compared to the present study. Although the methods used vary between studies, it can be noted that the results on MP ingestion in this study do not greatly differ from other studies conducted in the Baltic Sea.

In the present study, large regional variability was found in the ingestion of MP by coastal fish. The highest prevalence of MP was found in Kivinokka, Helsinki (27.5%) and in Porvoo (16.7%) which are both located on the southern coast of Finland, in the most densely populated county with a population density of 180 residents per km^2 in 2018 (National Land Survey of Finland, 2018; Statistics Finland, 2018). Kivinokka is situated near the large river of Vantaa and an intensely trafficked highway which are potential vectors and sources of MP. In Porvoo, the samples were taken near a cargo ship harbor and a large industrial area that supplies e.g. plastic materials. The other sampling sites, apart from Munkkiniemi, Helsinki are situated in other counties, where the population density is less than 50 residents per km^2 (National Land Survey of Finland, 2018, Statistics Finland, 2018). The prevalence of MP in fish in these sampling sites was less than 10%. These results are consistent with a previous study where more plastic litter was found in densely populated areas at 18 shoreline sites worldwide (Browne et al., 2011).

The number of MP in fish can, however, vary significantly even within relatively short distances. Only 5% of the fish in Munkkiniemi had ingested MP even though it is situated in the same city as Kivinokka but approximately 8.3 km apart. The variation may be explained by several factors. First, there is evidence suggesting that MP do not accumulate in the GITs of fish (Beer et al., 2018; Bråte et al., 2016; Güven et al., 2017). Thus, great variation in the numbers of ingested MP is possible even in the same sampling site over time (Rummel et al., 2016). Second, fish may encounter very patchy distributions of MP due to large temporal and spatial variation in the amount of plastic litter in sea water (Lusher et al., 2016; Ryan et al., 2009).

The sampling took place during the breeding season of bleak, stickleback and roach (Yrjölä et al., 2015). Perch breeds in shallow waters earlier in the spring and shifts into deeper waters as the spring progresses (Yrjölä et al., 2015). Thus, the perches caught in the shallow waters in May–June were likely not breeding. The prevalence of MP was lowest in the mainly benthic-feeding roach (2.4%) while the other three species feeding more in the water column had higher prevalence of MP. However, no statistically significant difference was found in the presence of MP in the fish species studied.

As also found by Foekema et al. (2013) and Güven et al. (2017), no relationship between the size of fish (length or weight) and prevalence of MP ingestion was observed. Although there was no statistical relationship between the fish size and the MP prevalence in fish, it is worth noting that MP occurrence in fish (stickleback > bleak > perch > roach) was found to be opposite to maximum size (stickleback < bleak < perch < roach). Although only fish of similar size were studied, perch were likely at younger stages than the other three species (Table 1). Thus, there is likely not much variation in the foraging strategies within the species, as the individuals of each species were roughly the same age. However, the role of size was not the focus of this study, since fish of similar size, regardless of species, were chosen, as the digestion method is best for only a few grams of wet tissue (Budimir et al., 2018), and heavier GITs could not have been digested as whole.

The size (largest diameter) of the MP found in this study was >100 μm. The size fraction <100 μm of the MP was most likely lost in the filtering process. The 100 μm mesh size was chosen, as the focus of the study was on the active uptake of MP, and the fish analysed in the present study feed on particles >100 μm (Yrjölä et al., 2015). However, fish may also ingest MP accidentally during normal feeding activity (Rummel et al., 2016), and thus using a smaller mesh size would likely have yielded a larger number of MP.

In the coastal water samples, the concentration of MP was higher (16.2 ± 11.2 MP m^{-3}) than in a previous study from the offshore area of the Gulf of Finland where less than 10 MP m^{-3} were found (Setälä et al., 2016). However, there was great variation between the coastal sampling sites. For example, no MP were found in Parainen whereas 33.9 MP m^{-3} were found in Munkkiniemi, Helsinki. Not only may potential local differences in the MP load to the coastal waters result in large variation between individual water samples, so may also the sampling technique. In the present study a pump was used for collecting water samples, which may result in variation due to patchiness of MP in water.

No statistical relationship was found between the presence of MP in the fish and concentration of MP in the water samples taken from the same locations. This can be partially explained by the uncertainty concerning the water samples described above. However, Lusher et al. (2016) also found no statistical relationship between the presence of MP in fish and water samples. On the other hand, Güven et al. (2017) did find more MP in fish in the sampling sites where MP were present in water and sediment.

More MP were found in both coastal fish and in coastal water samples compared to results from the offshore fish by Budimir et al. (2018) and offshore water samples by Setälä et al. (2016). Thus, a relationship between the amount of MP in the fish and in the water samples is detected on a larger scale. Due to the patchiness of MP in sea water and the temporal variation in MP ingestion by fish, this relationship might not be observable on a smaller scale between the different locations off the coastline.

5. Conclusions

Although the methods for tissue digestion and MP identification have further developed during the past few years, the method by Budimir et al. (2018) that was used in the present study offers an affordable and fast alternative for carrying out spatial scanning of microplastic exposure within different fish communities, which is beneficial for e.g. monitoring purposes. Qualitative methods, such as FT-IR or Raman spectra, were not used in the present study to further analyze the composition of MP. When available, using such methods likely improves the reliability of the results.

The present study provides the first record of plastics in the GITs of fish in coastal waters of Finland. A longer monitoring time over several years is recommended in order to get a more reliable assessment of the amounts of MP in fish.

CRediT authorship contribution statement

Erika Sainio: Investigation, Formal analysis, Data curation, Writing – original draft. **Maiju Lehtiniemi:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition. **Outi Setälä:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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